

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning on page 1, line 28 as follows:

Dependent on molecular weight, structure and doses, PEI can be toxic to certain cells. It has been reported that branched PEI with molecular weight (MW) of 25,000 Daltons or greater displayed relatively high cytotoxicity, possibly because of forming large aggregates on the surface of cells. Low molecular weight PEIs (< 1,800 Daltons) displayed much less toxicity but almost no transfection efficiency.

Please amend the paragraph beginning on page 2, line 32 as follows:

Typically, a polycondensation reaction occurs between a modified or activated cyclodextrin and a low molecular weight polyethylenimine. Hydroxyl groups in cyclodextrin are coupled to ~~amine~~ amine groups in polyethylenimine with the result of a one-carbon spacer.

Please amend the paragraph beginning on page 3, line 28 as follows:

By low molecular weight polyethylenimine, it is meant to include polyethylenimine having a molecular weight of less than about 25000 Daltons. The polyethylenimine monomers of choice typically have a molecular weight of less than about 20000 Daltons, preferably less than about 10000 Daltons, more preferably less than about 5000 Daltons. The present inventors have found that copolymers formed from polyethylenimine having molecular weights from about 500 to 4000 Daltons, preferably between about 600 to 2000 Daltons, are particularly suitable for delivering nucleic acid molecules to cells according to the present invention.

Please amend the section titled "Mode(s) of Carrying Out the Invention" on page 6, lines 1-15 as follows:

Mode(s) for Carrying Out the Invention

DEFINITIONS

PEI	polyethylenimine
PEI600	polyethylenimine having a molecular weight of 600 <u>Daltons</u>
PEI1200	polyethylenimine having a molecular weight of 1200 <u>Daltons</u>

PEI2000	polyethylenimine having a molecular weight of 2000 <u>Daltons</u>
PEI25k	polyethylenimine having a molecular weight of 25,0000 <u>25,000 Daltons</u>
CyD	cyclodextrin
β-CyD	β -cyclodextrin
PEI-CyD	polyethylenimine / cyclodextrin copolymer
PEI600-CyD	polyethylenimine 600 / cyclodextrin copolymer
PEI1200-CyD	polyethylenimine 1200 / cyclodextrin copolymer
PEI2000-CyD	polyethylenimine 2000 / cyclodextrin copolymer
CDI	1,1'-carbonyldiimidazole
CDI-CyD	β -cyclodextrin-carbonate-benzotriazole

Please amend the paragraph beginning on page 6, line 19 as follows:

Polyethylenimine (PEI), average molecular weight 600, 1200, and 2000 Daltons, β -cyclodextrin (CyD, molecular weight 1,135) and 1,1'-carbonyldiimidazole (CDI) were used for copolymer synthesis. 0.42 g β -cyclodextrin (0.00037 mol) and 0.80 g 1,1'-carbonyldiimidazole (0.0052 mol) were dissolved in 6 ml N,N-dimethylformamide (DMF). The mixture was stirred at room temperature and reacted for 1h. Reaction system was protected by nitrogen. The mixture was precipitated in cold ethyl ether, filtered, dissolved in 5 ml dimethylsulfoxide DMSO, stored at 4°C. PEI polymers (1.5 g, 0.0025 mol) were dissolved in 3 ml DMSO. After PEI dissolved in the reaction solution, CyD-CDI in 5 ml DMSO and 0.3 ml triethylamine (Et₃N) was added drop wise over 1.5h. After additional stirring for 4-5h, the mixture was dialyzed in water and freeze-dried for 2 days.

Please amend the paragraph beginning on page 7, line 11 as follows:

Molecular weights of synthesized copolymers were determined by GPC using Waters 600E pump and Waters 410 Refractive Index Detector (33°C). The columns were Phenomenex Polysep Guard S/n 70978G, Polysep GFC-P S/n 70977 and Polysep GFC-P S/n 70976 (33°C). PEI600-CD and PEI2000-CD samples were run at a 10 mg/ml concentration in distilled-water.

The running solution used was distilled water, with a flow rate of 0.8 ml/min. The injected volume was 300 μ l. Molecular weight analyses were made against five poly(ethylene glycol) standard of number average molecular weights 7100, 10600, 12600, 23600 and 56000 Daltons.

Please amend the paragraph beginning on page 10, line 26 as follows:

Mass spectral analysis of PEI2000-CyD showed dominant peaks at MW 3900 to 4000 Daltons and confirmed the linkage of PEI2000 and cyclodextrin (Figure 5). The GPC analysis indicated that the molecular weights increased from 600 to 63,000 Daltons and 2000 to 19,000 Daltons, respectively (Figure 6), confirming the cross-linking of the low molecular weight PEIs by CyD. In one preparation, the molecular weight of PEI2000-CyD reached 40,000 Daltons.

Please amend the paragraph beginning on page 11, line 29 as follows:

PEI-CyD copolymers developed according to the present invention undergo hydrolytic degradation in PBS (pH 7.4) at 37°C, because of ester bonds formed. Within 25 days, the molecular weight of the copolymer decreased from 63000 to 30000 Daltons. With the degradation, plasmid DNA was released from copolymer complexes. The release rate of DNA was a function of charge ratio. Complexes at the N/P ratio of 1 started to release DNA after 2 days of incubation in PBS at 37°C as indicated by agarose gel analysis (Figure 12). Higher charge ratios lead to slower release of DNA from the complexes. At the N/P ratio of 6, DNA release started to appear at day 3. The electrophoretic mobility of DNA release from the complexes was slightly lower, probably because of the effects of the degraded low molecular weight PEI600 oligomers. As well, the viscosity analysis of the copolymer dissolved in PBS showed a 60% decrease within 36 days (Figure 13).

Please amend the paragraph beginning on page 12, line 24 as follows:

The present invention provides biodegradable copolymers, synthesized through low molecular weight polyethylenimine (typically less than about 2,000 Daltons) cross-linked by modified β -cyclodextrin. The terminal β -cyclodextrin hydroxyl group was activated by 1,1'-

carbonyldiimidazole. Each activated β -cyclodextrin links two or three units of low molecular weight PEI to form a linear structure copolymer.

Please amend the paragraph beginning on page 12, line 29 as follows:

Modification degree (%) of β -cyclodextrin and 1,1'-carbonyldiimidazole were calculated by proton NMR, showing 33% of hydroxyl group in β -cyclodextrin being modified. Activated β -cyclodextrin was reacted with low molecular weight PEI to form linear structure of PEI-CyD. Ester bonds were formed during the reaction of activated CyD and PEI. GPC measurement showed that the molecular weight of the synthesized copolymers increased from 600 to 23,000 Daltons and 2,000 to 19,000 Daltons for PEI600-CyD and PEI2000-CyD, respectively. Typically the copolymers of the invention contain 10 or 15 PEI-CyD units.